

# **Report on the 2008 Proficiency Test of the Community Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories, regarding the Determination of Deoxynivalenol in a Cereal Product and a Test Solution**

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## Summary

A proficiency test was conducted by the Community Reference Laboratory for Mycotoxins with 33 European National Reference Laboratories (NRLs) for Mycotoxins and 2 Laboratory from candidate countries, thus a total of 35 participants. Test materials were a deoxynivalenol (DON) solution in acetonitrile and three cereal test materials. Laboratories determined the DON content by either enzyme linked immuno sorbent assay (ELISA), gas chromatography (GC) or reverse-phase high-performance liquid-chromatography (RP-HPLC). One NRL did not report any results.

Applying the Horwitz equation as a basis for the target standard deviation (19% in the case of this proficiency test), 27 out of the remaining 34 laboratories reported values within the z-score limit of 2 after recovery correction of the result for the DON-positive sample. Twenty-five laboratories reported results within a z-score limit of 1. Thus, 79 % of the participating laboratories performed satisfactorily in the proficiency test. No z-scores were calculated for the blank material.

## Introduction

In 2006 the Institute for Reference Materials and Measurements (IRMM) in Geel was designated as Community Reference Laboratory (CRL) for Mycotoxins by the Directorate General for Health and Consumers (DG SANCO). One of the main responsibilities of the CRL is to organise comparative testing to benchmark and harmonise the measurement capabilities of National Reference Laboratories (NRLs) working in the same field. Therefore, the CRL for Mycotoxins together with the network of NRLs agreed to conduct the proficiency test (PT) in 2008 (PT2008) as follow up action to the PT it organised in 2006 and 2007. The topic of the PT2008 was the determination of deoxynivalenol (DON) in cereals.

Test materials in this study were maize based cereal flours, either free or naturally contaminated with DON. The batch was tested for homogeneity using an ANOVA based experimental design and found sufficiently homogeneous. The stability of the test material was not tested explicitly, as the material was intended to be used shortly after preparation in the PT and previous studies on the fate of DON in dry cereal based products did not indicate any reason for the need of short term stability testing.

## Methodology

Each participant received one ampoule containing the DON test solution and three cereal materials;

- 2 coded test materials with a level of DON unknown to the participants of which one contained DON at a level less than 20 ng/g and was considered as blank in the following
- 1 material marked as "blank" for recovery experiment (<20 ng/g DON).
- 1 ampoule of a test solution of "DON in acetonitrile" with a concentration of DON unknown to the participants.

Participants were asked to measure the two coded cereal test materials and the test solution for DON, and to spike the blank cereal material with their own calibrant, reporting the spiking level and amount found to obtain recovery information. The detailed instructions as sent to the participants are included in the annex.

All graphs were made with SigmaPlot 9.01. Results were gathered via electronic forms using Adobe Life-cycler. Z-scores were calculated using Microsoft Excel®.

As basis for the z-scoring, the predicted Horwitz standard deviation was used, which was in this case 19% (z-score = 1)

## Results and Discussion:

### *Assignment of consensus values*

The DON test solution was produced from a Certified Reference Material (CRM) calibrant solution by dilution (IRMM-315). The assigned value of the prepared test solution (the coverage factor  $k=2$  corresponding to a level of confidence of about 95 %) was  $0.304 \pm 0.015 \mu\text{g/mL}$ . A full report on the production and certification of the initial CRM calibrant, which was used to prepare the calibrant solution, is available from the IRMM<sup>1</sup>.

For the DON positive test material, which was naturally contaminated, the median of the values submitted by the laboratories was chosen as the assigned value. This is a rather straight forward and easy method and does not significantly differ from results calculated by other methods, such as robust means (see Table 1). Prior computation extreme values have been taken out (Lab-ID 133).

**Table 1:** Calculation of assigned values<sup>2</sup>:

Parameter	Uncorrected result [ $\mu\text{g/kg}$ ]	Recovery corrected result [ $\mu\text{g/kg}$ ]
Median	283	304
A15 mean	287	306
H15 mean	290	307

For each tested material the individual z-scores (cereal material) respectively the %-deviation from the assigned value (test solutions) are listed together with all single results submitted by the participants in **Table 2 - 3** in the annex.

**Figure 1** depicts the ranking of the results of the participating laboratories for DON in the cereal product (containing DON) prior recovery correction and prior calibrant correction, in ascending order. The assigned value is depicted by a black line. The limit for a z-score of  $z = |2|$  is indicated by red lines. For 26 of the laboratories the reported value fell within the z-score limit of 2. Eight laboratories reported values outside this limit. No further evaluation was made on this result, as only the recovery corrected result is of relevance for evaluation. The assigned value was calculated out of the median of all submitted results with the exception of the result from participant ID 133.

### *Correction for recovery*

The effect of recovery correction on the results can be seen in **Figure 2**. As a result of this, 27 of the laboratories had values within the z-score limit of 2. Seven laboratories reported values outside this limit. Consequently, no improvement of the overall population performance based on the  $z = |2|$  limit can be observed by taking into account recovery. However, on a  $z = |1|$  limit an improvement can be observed as 22 laboratories fell within that limit prior recovery correction, whereas 25 did after correction. The assigned value was calculated out of the median of all submitted results (after recovery correction) with the exception of the result from participant ID 133.

<sup>1</sup> <https://irmm.jrc.ec.europa.eu/rmcatalogue/detailsrmcatalogue.do?referenceMaterial=I-0315%2B%2B%2B%2B%2B%2B>

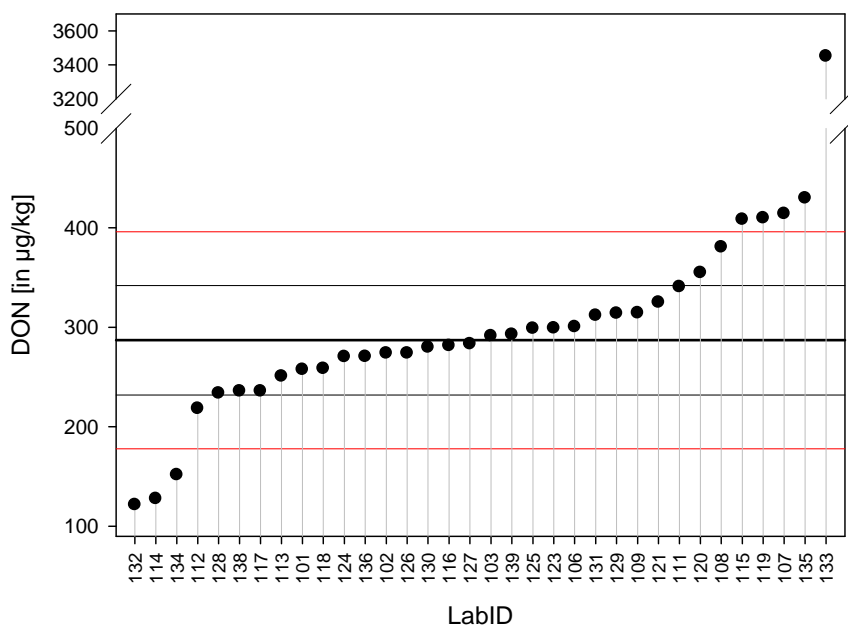
<sup>2</sup> A15 and H15 means have been calculated according to "Analytical Methods Committee, Analyst, 1989, 114, 1489", using an excel sheet available from the Royal Chemists Society. See here also for more details on these parameters.

### Measurement of the test solution

The dispersion of results concerning the test solution is shown in **Figure 3**. As there is little information or agreement how to set a target standard deviation for the measurement of pure calibrants, no such figure was used for calculating z-scores. However, it can be assumed that the simple measurement of a test solution should give a smaller dispersion of results than the results for the test material that require a more complex analytical treatment for generation. Due to lack of any agreed procedure, results are calculated in **Table 3** as deviation from the assigned value. Under the above mentioned assumption that this type of measurement is simpler and thus much less influenced by analytical procedures it can serve as a good indicator for the quality of the laboratory calibrant used in this PT.

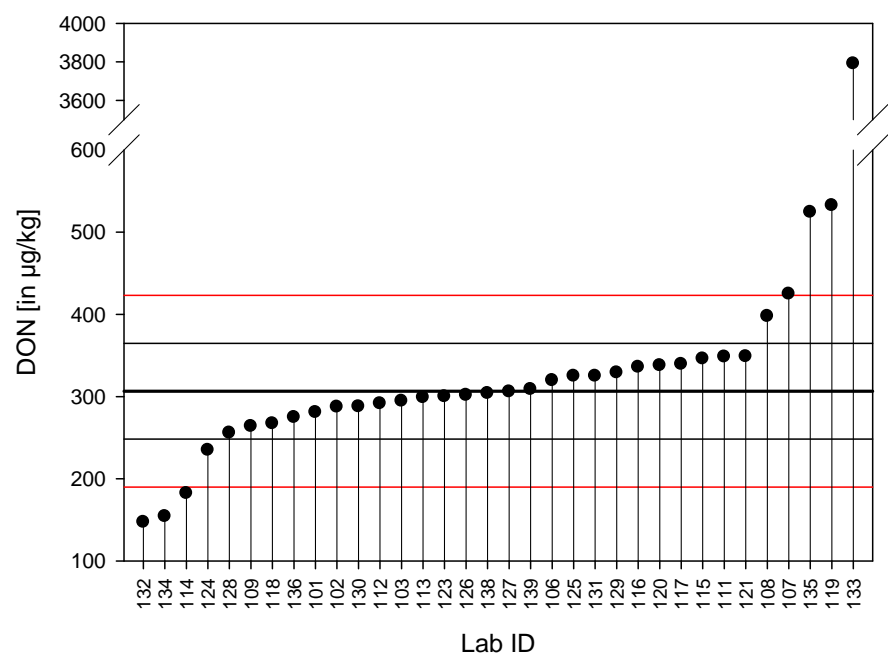
An important aspect to consider is that there are only limited possibilities for routine laboratories to confirm the concentration and purity of DON calibrants (unlike for aflatoxins<sup>3</sup>). Thus, laboratories largely rely on the manufacturers' statements and, once prepared, on the stability of the solutions in their laboratory. Participants that reported results far outside the uncertainty range of the test solution, shall investigate the exact source for the deviation.

**Figure 1:** Plot of DON results in the naturally contaminated cereal product prior to recovery correction. The bold black line reflects the assigned value, red lines the z-score limit of  $z=|2|$ .

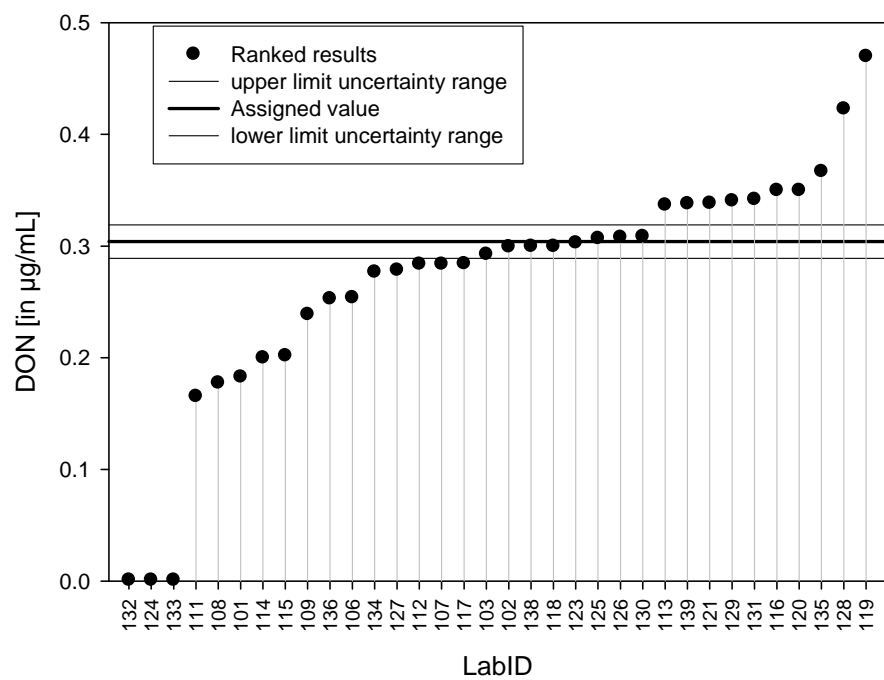


<sup>3</sup> See previous NRL PT reports from 2006 and 2007

**Figure 2:** Plot of DON results in the naturally contaminated cereal product after recovery correction. The bold black line reflects the assigned value, red lines the z-score limit of  $z=|2|$ .



**Figure 3:** Plot of DON results in the test solution. The bold black line reflects the assigned value, the thin black lines the uncertainty range of the concentration of the solution.





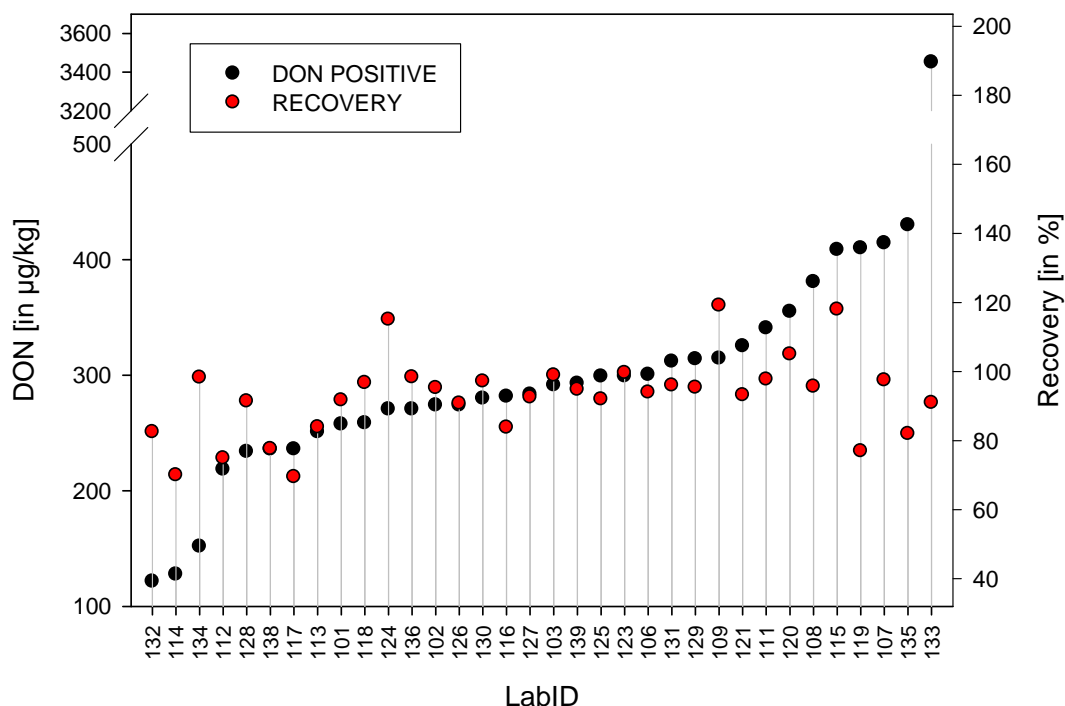
### *Correlation between recovery and uncorrected value*

One parameter to examine proper recovery estimation is to correlate the recovery with the recovery-uncorrected result for the cereal test material. This correlation can be regarded as an indicator whether the reported recovery values have been correctly estimated under the test conditions applied by the participants in this PT. Both scales (left legend = uncorrected result for the test material; right legend = reported recovery) are scaled so that 100% recovery matches with the assigned value from the recovery corrected result (**Figure 4**).

If the reported recovery rate and the recovery-uncorrected result are displayed in close proximity to each other in Figure 4 (red and black points), the correction for recovery of the result of the test material has a positive effect on the z-score as the correction improves the result. In case that recovery values were incorrectly estimated the scenario inverses resulting in enlarged z-scores after recovery correction. As an example, results improve for Lab-ID 112 after recovery correction compared with the recovery uncorrected result (**Figure 1** → **Figure 2**) due to the well corresponding values for both results. This is the opposite for Lab-ID 124, where a previously good z-score got poorer after recovery correction.

This highlights the importance of correctly estimating recovery values and in addition might set the basis for the discussion how recovery values should be generated to improve analytical results. One aspect that becomes apparent is that in general the dispersion of recovery values is less than the one for the unknown sample. This could indicate that recovery estimates are much more optimistic (with regards to a target value of 100%) than they are in reality, assuming that a correct correction for recovery should lead harmonised values. One explanation for this might be that for "blind" measurements no target value is known and thus this value is subject to acceptance "as is". For recovery values a target value exists (usually 100%) and any large deviation (even if reflecting the correct performance of the method at that particular moment) might be subject to doubt. As a result repetition and/or correction is made until a sufficient closeness to the expected target value is achieved.

**Figure 4:** Correlation between recovery rate and recovery-uncorrected value for the DON positive sample. Black points (●) display the amount reported for the uncorrected DON positive material (left ordinate) and red points (●) the reported recoveries. Both ordinates are scaled to match the recovery corrected consensus value with a 100 % recovery.



### *Evaluation of the comments in the questionnaire*

With few exceptions the pool of participants had more than two years of experience in the analysis of DON. Concerning the procedures applied the following comments should be noticed. In some cases participants reported that the analysis was carried out within two consecutive days which means that the analysis was interrupted (over night) at various stages (LabID 111, 118, 124, 128, 138 and 139). No effect, such as general under or overestimation, of the result could however be attributed to such an interruption. This indicates that clean-up and chromatography can be performed at different days, without negatively affecting the results. Even a storage of the extracts (MeCN-water) for a 6 weeks period resulted in acceptable results (LabID 124).

Most laboratories used peak area for signal reporting and valley-to-valley integration settings in combination with visual inspection to verify correctness of integration. Most integration settings can be regarded as robust, as only in a few cases chromatograms needed to be manually re-integrated. The reported number of re-integrated peaks was around 1 - 4 for the whole set of analyses; in once case up to 20. As no further information was given on these circumstances, the need for visual inspection can only be a recommendation. In the case of 20 re-integrations (LabID 115) this figure demonstrates the minimum amount of chromatograms generated for this PT. As long as these re-integrated chromatograms refer to the overall measurements (including calibration) this is acceptable from the point of the amount of work that was dedicated to the analysis of the PT materials.

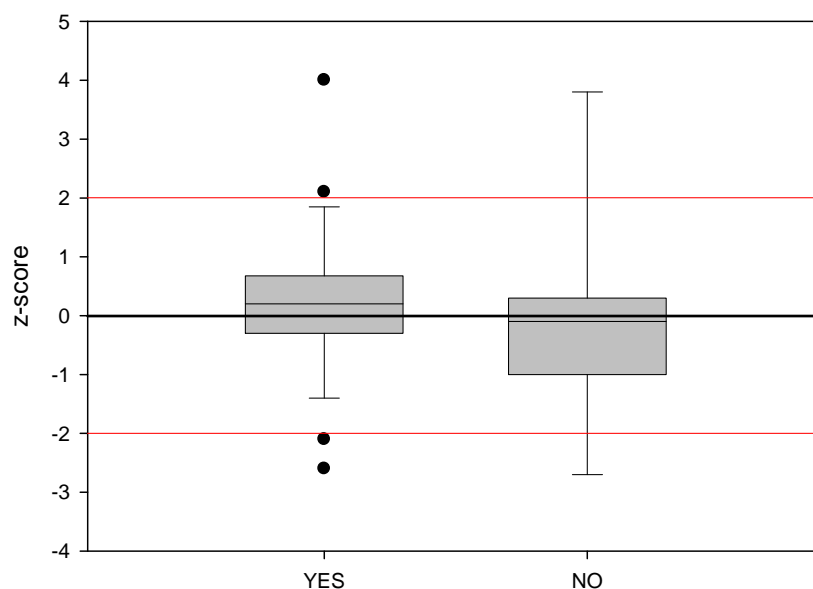
Most participants found the instructions and the e-Form for reporting adequate, however a few reported problems or mentioned short-comings, which were however not always qualitatively described. All comments will be taken into consideration for design of documents/and procedures in the next PT.

Laboratories were asked to report if they are accredited according to ISO 17025 for this type of analysis and both pools (accredited and not accredited) were compared in a box plot (**Figure 5**). Independently of the status of accreditation, the two pools performed similarly, not allowing for any

conclusion on the possible correlation between the status of accreditation and the quality of the reported result.

One important comment was to allow the participants more freedom in the way of producing recovery values.

**Figure 5:** Comparison of status of accreditation for the tested parameter

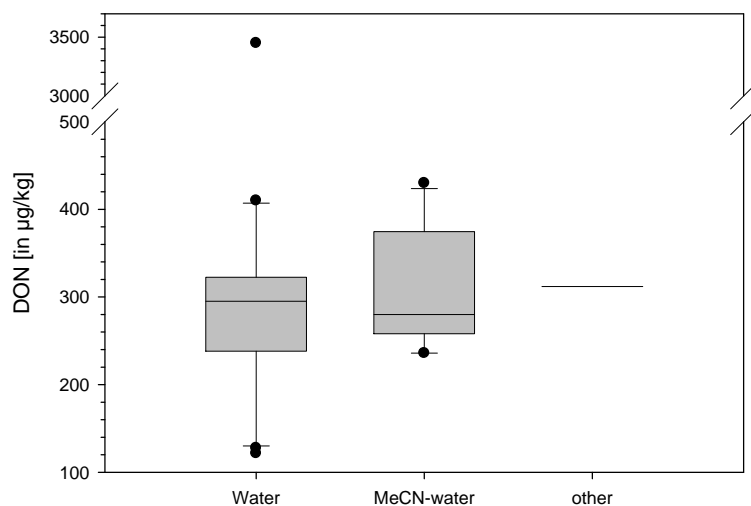


The left box-plot shows the dispersion of results for accredited laboratories (YES) and the right box-plot that of those not accredited for DON. Red horizontal lines indicate the z-score limit of 2. Result of LabID 133 is not displayed.

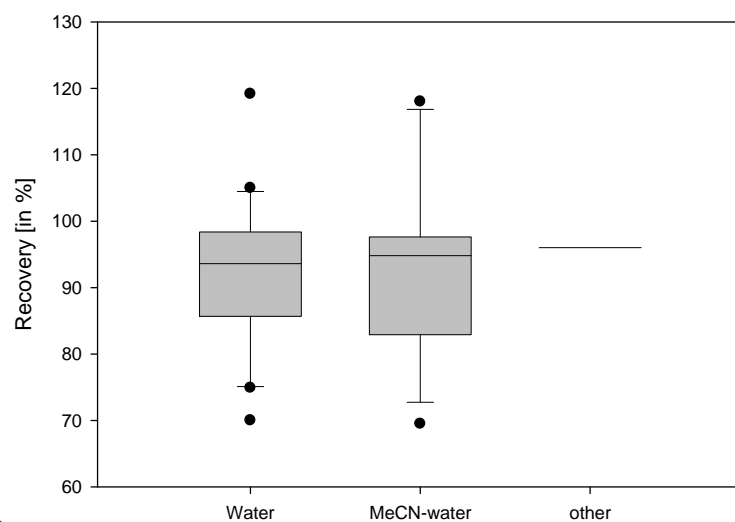
### *Comparison of methodological parameters*

In addition to the information on the estimation of the performance of a participant, a PT can under certain circumstance offer additional information on methodological parameters such as extraction, clean-up, etc. In the following, different parameters are grouped and compared. The comparisons are visualised with Box and Whisker plots. As an example **Figure 6** shows the comparison of the main two different extraction solvents that are commonly used for the analysis of DON. Apparently water, as an extraction solvent is comparable to MeCN-water mixtures.

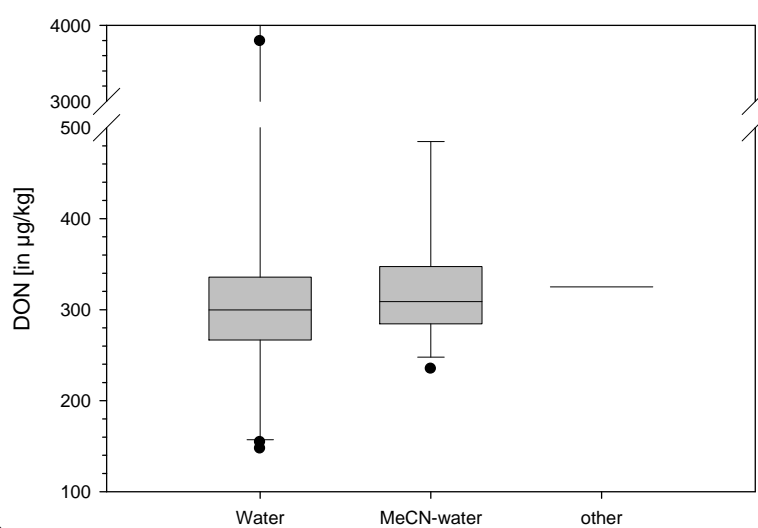
**Figure 6:** Box and Whisker plots of different extraction solvents used by participants of the PT. The data refer to the uncorrected value of DON (a), the recovery reported (b), and the recovery corrected results (c).



6a)



6b)

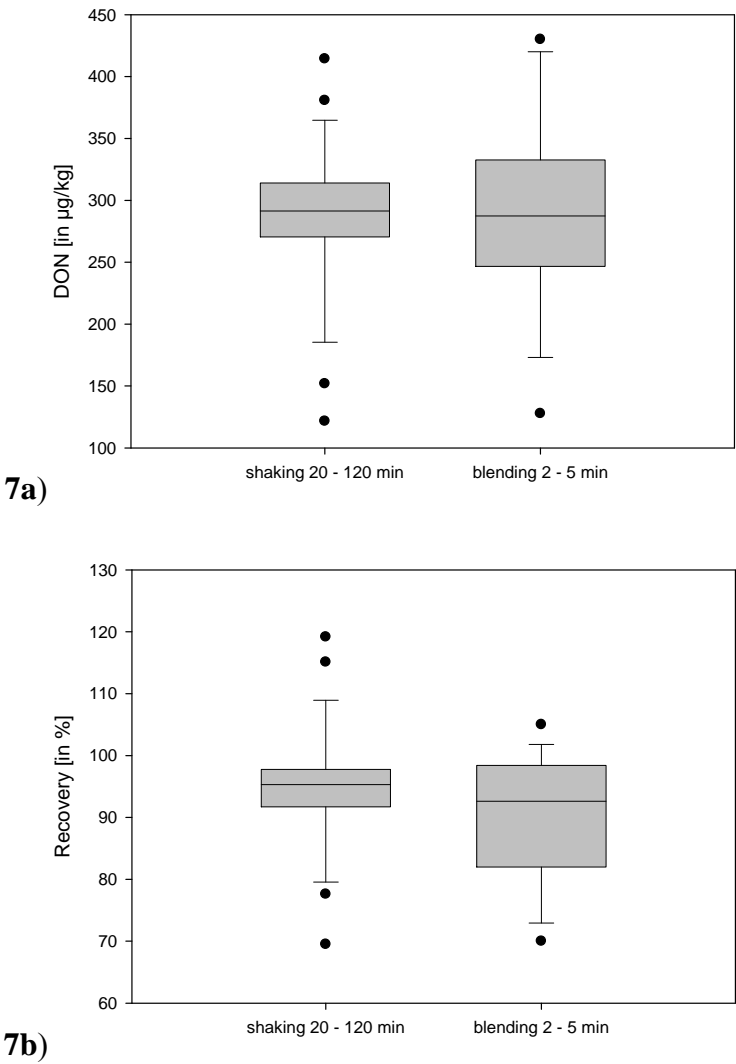


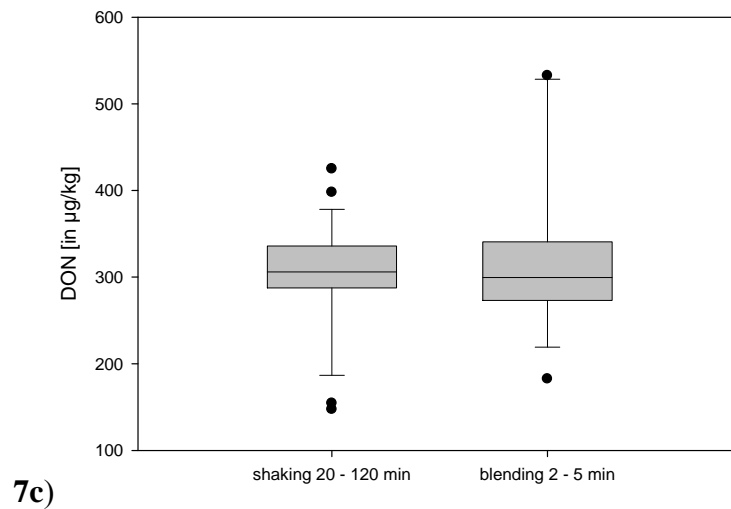
6c)

Another parameter that apparently varies in laboratories is the extraction mode by either shaking or blending. Shaking requires a longer time, but offers the processing of several samples simultaneously and minimises the risk of cross contamination. Blending is often preferred when just a low number or

single samples need to be analysed. A comparison of data shows (**Figure 7**) that both extraction principles are equivalent.

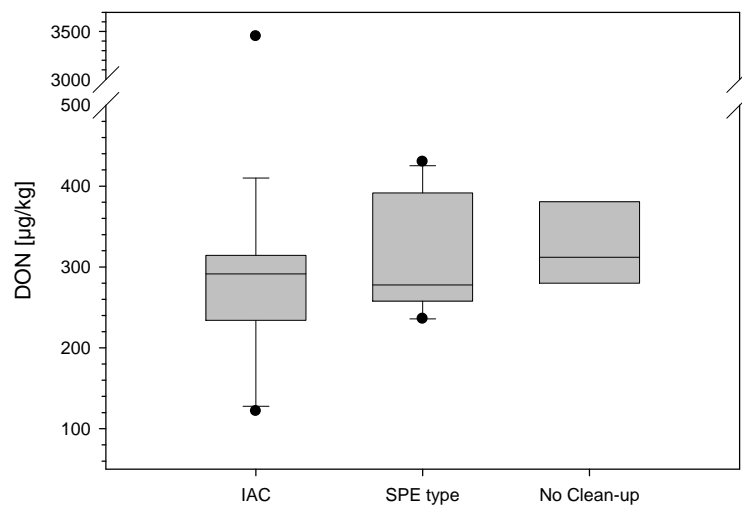
**Figure 7:** Box and Whisker plots illustrating the effect of different extraction procedures used by participants of the PT on the measured DON values. The data refer to the uncorrected value of DON (6a), the recovery reported (6b) and the recovery corrected result (6c). In the top and bottom chart results from Lab 133 were taken out as they were far out of scale ( $>3000\text{ }\mu\text{g/kg}$ ).



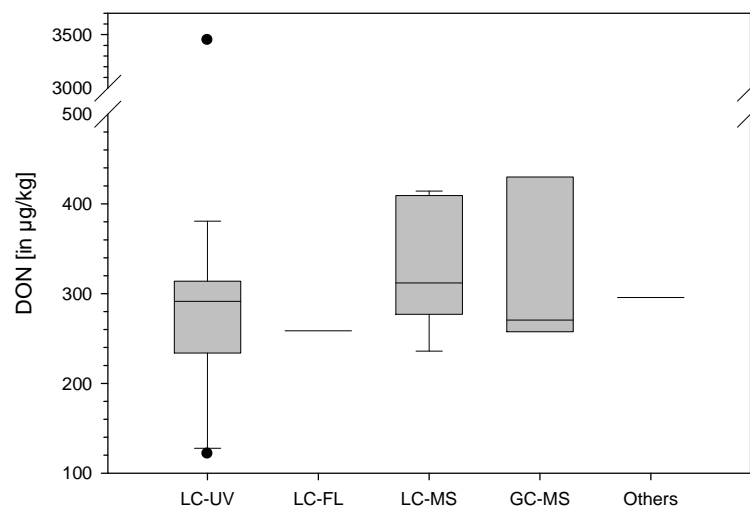


Other interesting parameters to compare are the clean-up procedures (**Figure 8**) or the determination methodology used (**Figure 9**). **Figure 8** indicates that the three clean-up procedures used (immunoaffinity clean-up, solid phase extraction or even no clean-up) achieve similar results. The same applies to the comparison of detection methodologies (**Figure 9**).

**Figure 8:** Comparison of different clean-up procedures on the uncorrected DON result.



**Figure 9:** Comparison of detection methodology used on the uncorrected result:



## Conclusion

According to the results obtained during this proficiency test,

- 79 % of the participating laboratories are able to determine DON in cereal in a satisfactory manner;
- The importance of recovery correction for the harmonisation of results could be demonstrated by putting recovery uncorrected results with the reported recovery into correlation.
- During extraction, water performs equally well as MeCN-water mixtures, and shaking performs equally well to blending.
- All three types of detection methodology applied by the laboratories performed equally well (LC-UV, LC-MS and GC-MS).
- Non-accredited laboratories performed equally well to accredited laboratories.
- It is proposed that the seven participants whose z-scores exceeded an absolute value of 2 participate in a follow-up PT for DON in cereals.

## Acknowledgments:

The authors wish to thank Anne-Mette Jensen, Piotr Robouch, Pieter Dehouck and Franz Ulberth for valuable comments.

# Annex

**Table 2:** Individual results as reported by the participants:

Laboratory code	PMC	PM	BLC	BL	TS	SP+	SPF	REC
101	D-030	257.6	D-130	10.3	0.183	300.0	275.0	91.7
102	D-024	274.0	S-111	0.0	0.2995	199.4	190.0	95.3
103	D-023	291.4	D-115	>50.0	0.293	200.0	197.7	98.9
106	D-004	300.5	D-106	>70.0	0.254	250.0	235.0	94
107	D-035	414.3	D-122	29.3	0.284	200.0	204.9	97.5
108	D-012	380.7	D-114	26.9	0.178	200.0	191.4	95.7
109	D-037	314.5	D-116	84.9	0.239	300.0	357.5	119.17
111	D-032	340.7	D-127	0.0	0.1656	300.0	293.3	97.77
112	D-050	218.5	D-110	0.0	0.284	262.5	196.5	74.9
113	D038	250.9	D145	0.0	0.337	257.2	215.7	83.9
114	D021	127.7	D119	0.0	0.200	272.0	190.3	70
115	D-049	408.5	D-144	4.6	0.202	100.0	118.0	118
116	D-027	281.6	D-129	6.9	0.350	290.8	243.9	83.8
117	D-045	236.0	D-121	0.0	0.284	300.0	208.5	69.5
118	D-026	258.6	D-120	2.2	0.300	190.4	184.2	96.74
119	D- 041	410.0	D-139	143.9	0.470	288.0	223.0	77
120	D-011	355.0	D-118	13.0	0.350	200.0	209.9	105
121	D-018	325.2	D-102	7.2	0.3385	209.1	194.9	93.2
123	D-033	299.2	D-131	8.3	0.303	200.8	200.1	99.65
124	D-046	270.5	D-140	9.0	0.000	250.0	287.7	115.1
125	D22	299.0	D124	6.6	0.307	300.0	277.0	92
126	D-015	274.0	D-108	>25.0	0.308	229.0	208.0	90.8
127	D-044	283.4	D-109	0.0	0.2787	200.4	185.5	92.6
128	D031	233.9	D113	0.0	0.423	199.7	182.6	91.4
129	D-020	314.0	D-104	0.0	0.341	250.0	238.5	95.4
130	D-040	280.0	D-126	0.0	0.3087	100.0	97.2	97.2
131	D-025	312.0	D-117	0.0	0.342	300.0		96
132	D-036	121.6	D-125	0.0	0.000	160.0	132.0	82.5
133	D-009	3451.0	B-073	157.0	0.000	200.0	182.0	91
134	D-028	151.8	D-103	0.0	0.277	150.0	147.4	98.3
135	D-039	430.0	D-041	30.0	0.367	300.0	246.0	82
136	D017	270.6	D107	0.0	0.253	170.0	167.3	98.41
138	D-005	235.9	D-112	0.0	0.300	172.8	134.1	77.6
139	D-010	292.8	D-132	0.0	0.3381	217.4	206.1	94.80

**PMC** = DON positive material container code, **PM** = DON positive material [ $\mu\text{g/kg}$ ], **BLC** = DON coded blank container code, **BL** = DON blank [ $\mu\text{g/kg}$ ], **TS** = test solution [ $\mu\text{g/mL}$ ], **SP+** = spiking level [ $\mu\text{g/kg}$ ], **SPF** = spike recovered [ $\mu\text{g/kg}$ ] and **REC** = recovery value [%].



**Table 3:** Performance parameters for the recovery corrected DON result and the test solution:

<b>Laboratory code</b>	<b>z-score</b>	<b>%-deviation test solution</b>
101	-0.4	-39.8
102	-0.3	-1.5
103	-0.2	-3.7
106	0.3	-16.4
107	2.1	-6.6
108	1.6	-41.6
109	-0.7	-21.4
111	0.8	-45.5
112	-0.2	-6.6
113	-0.1	10.9
114	-2.1	-34.2
115	0.7	-33.6
116	0.6	15.1
117	0.6	-6.4
118	-0.6	-1.3
119	4.0	54.6
120	0.6	15.1
121	0.8	11.3
123	-0.1	-0.3
124	-1.2	-99.7
125	0.4	1.0
126	0.0	1.3
127	0.0	-8.3
128	-0.8	39.1
129	0.4	12.1
130	-0.3	1.5
131	0.4	12.5
132	-2.7	-99.7
133	60.4	-99.7
134	-2.6	-8.9
135	3.8	20.7
136	-0.5	-16.8
138	0.0	-1.3
139	0.1	11.2

The calculated z-scores refer to the **recovery corrected value**. Underreported values for the content of the test solutions are indicated as negative figures.

**Table 4a:** Evaluation of the Questionnaire:

Lab ID\ Question	Analytical experience with DON determination	Time of experience	Number of samples per year	Evaporation time [minutes]	Spiking solvent
101	YES	6-12 months	51-100	60	MeCN
102	NO	1-5 months	05-050	30	MeCN
103	YES	> 2 years	101-500	40	MeCN
106	YES	> 2 years	51-100	120	other
107	YES	> 2 years	101-500	20	MeCN
108	YES	1-2 years	101-500	10	MeOH
109	YES	6-12 months	05-050	15	MeCN
111	YES	> 2 years	>500	1000	MeCN
112	YES	> 2 years	51-100	30	MeCN
113	YES	> 2 years	05-050	45	MeCN
114	YES	> 2 years	51-100	1	Water
115	YES	> 2 years	05-050	60	MeCN
116	YES	> 2 years	05-050	30	MeCN
117	YES	1-2 years	101-500	1	MeCN
118	YES	> 2 years	51-100	30	MeCN
119	YES	> 2 years	05-050	60	MeCN
120	YES	> 2 years	101-500	60	MeCN
121	YES	> 2 years	101-500	45	MeCN
123	YES	> 2 years	51-100	20	MeOH
124	YES	> 2 years	51-100	10	MeCN
125	YES	1-2 years	51-100	30	other
126	YES	> 2 years	>500	30	MeCN
127	YES	1-2 years	05-050	720	MeOH
128	YES	1-2 years	05-050	30	mobile phasis (water/MeOH/ACN)
129	YES	> 2 years	101-500	90	other
130	YES	> 2 years	>500	60	MeCN
131	YES	> 2 years	101-500	15	MeOH
132	NO	xxx	05-050	180	other (ETHANOL)
133	YES	> 2 years	05-050	60	MeCN
134	YES	> 2 years	05-050	30	other
135	YES	> 2 years	05-050	30	MeCN
136	YES	> 2 years	51-100	180	other
138	YES	> 2 years	51-100	30	other, chloroform
139	YES	> 2 years	51-100	30	MeCN

**Table 4b:** Evaluation of the Questionnaire:

Lab ID\ Question	Have there been any over night stops in the course of analysis?
101	no
102	no
103	no
106	no
107	no
108	no
109	no
111	for all solid samples; between clean up and silylation
112	no
113	no
114	no
115	no
116	no
117	no
118	The final extracts of samples D - 120 and D - 026 were kept overnight (in refrigerator)and injected the following day.
119	D-139 after extraction
120	no
121	no
123	no
124	All samples. Due to the instrumental problems (that were observed only after summer vacations), the whole set had to be re-analysed 6 weeks after the original extraction. During this time, all extracts had been stored at +4 degrees.
125	no
126	no
127	After spiking samples were left overnight to evaporate
128	Extraction solutions were kept overnight before application to IA-columns
129	no
130	no
131	no
132	no
133	no
134	no
135	no
136	no
138	HPLC analysis performed the day after preparation for all samples
139	Day 1: extraction, clean-up, evaporating to dryness Day 2: dissolving in the mobile phase, HPLC/UV running

**Table 4c:** Evaluation of the Questionnaire:

Lab ID\ Question	Methodology	Clean-up	Detector	Extraction mode
101	GC	MycoSep	Mass Spec (Single Quad)	Shaking
102	LC	Immunoaffinity	UV-absorption	Shaking
103	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
106	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
107	LC	MycoSep	Mass Spec (Triple Quad)	Shaking
108	ELISA	No clean-up	UV-absorption	Shaking
109	LC	Immunoaffinity	UV-absorption	Shaking
111	GC	MycoSep	Other = ECD	Other = stirring
112	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
113	Other	Immunoaffinity	Other	Blending (e.g. Turrax)
114	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
115	LC	Other	Mass Spec (Triple Quad)	Other
116	LC	MycoSep	Mass Spec (Triple Quad)	Shaking
117	LC	MycoSep	Mass Spec (Triple Quad)	Shaking
118	LC	MycoSep	Fluorescence	Blending (e.g. Turrax)
119	LC	Immunoaffinity	Mass Spec (Single Quad)	Blending (e.g. Turrax)
120	LC	Immunoaffinity	Mass Spec (Triple Quad)	Blending (e.g. Turrax)
121	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
123	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
124	GC	MycoSep	Mass Spec (Single Quad)	Shaking
125	LC	Immunoaffinity	UV-absorption	Shaking
126	LC	Other	Mass Spec	Shaking
127	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
128	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
129	LC	Immunoaffinity	UV-absorption	Shaking
130	LC	No clean-up	Mass Spec (Triple Quad)	Shaking
131	LC	No clean-up	Mass Spec (Triple Quad)	Shaking
132	LC	Immunoaffinity	UV-absorption	Shaking
133	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
134	LC	Immunoaffinity	UV-absorption	Shaking
135	GC	Dispersive "SPE"	Mass Spec (Single Quad)	Blending (e.g. Turrax)
136	LC	Immunoaffinity	UV-absorption	Blending (e.g. Turrax)
138	LC	Other, MultiSep and immunoaffinity	UV-absorption	Shaking
139	LC	MycoSep	UV-absorption	Shaking

**Table 4d:** Evaluation of the Questionnaire:

Lab ID\ Question	Extraction time	Extraction solvent	Extraction solvent ratio	Overnight steps	Problems during analysis
101	60	MeCN-Water	84:16	NO	NO
102	20	Water only	pure	NO	NO
103	3	Water only		NO	NO
106	3	Water only	pure	NO	NO
107	60	MeCN-Water	84:16	NO	YES
108	10	Water only	pure	NO	YES
109	30	Water only		NO	NO
111	120	MeCN-Water	84:16	YES	NO
112	3	Water only	pure	NO	NO
113	3	Water only		NO	NO
114	2	Water only		NO	NO
115	60	MeCN-Water	84:16	NO	NO
116	120	MeCN-Water	84:16	NO	NO
117	60	MeCN-Water	84:16	NO	NO
118	3	MeCN-Water	84:16	YES	NO
119	2	Water only	pure	YES	NO
120	3	Water only	pure	NO	NO
121	3	Water	PEG	NO	NO
123	3	Water only	xxx	NO	NO
124	120	MeCN-Water	84:16	YES	YES
125	30	Water only		NO	NO
126	120	MeCN-Water	86:14	NO	YES
127	2	Water only		YES	NO
128	2	Water only		YES	NO
129	120	Water only	pure	NO	NO
130	120	MeCN-Water	80:20	NO	NO
131	120	Other	other	NO	NO
132	0	Water only	pure	NO	NO
133	3	Water only		NO	NO
134	30	Water only		NO	NO
135	5	MeCN-Water	84:16	NO	NO
136	2	Water only		NO	NO
138	30	MeCN-Water	84:16	YES	NO
139	60	MeCN-Water	84:16	YES	NO

**Table 4e:** Evaluation of the Questionnaire:

Lab ID\ Question	observations	Signal reporting	Integration by	Visual integration check	# of reintegrated peaks	Integration settings
101	NO	PEAK SIGNALS	AREA	NO		horizontal baseline
102	NO	PEAK SIGNALS	HEIGHT	YES	6	valley-to-valley
103	NO	PEAK SIGNALS	AREA	YES	0	Horizontal baseline
106	NO	PEAK SIGNALS	AREA	YES	0	valley to valley
107	YES	PEAK SIGNALS	AREA	YES	2	valley-to-valley
108	NO	ABS (ELISA)		NO	0	xxx
109	NO	PEAK SIGNALS	AREA	NO	0	xxx
111	NO	PEAK SIGNALS	HEIGHT	YES	0	valley-to-valley
112	NO	PEAK SIGNALS	AREA	YES	0	Empower (Waters) "Apex" calculation
113	NO	PEAK SIGNALS	AREA	YES	3	
114	NO	PEAK SIGNALS	HEIGHT	xxx	0	xxx
115	NO	PEAK SIGNALS	AREA	YES	20	valley-to-valley
116	NO	PEAK SIGNALS	AREA	YES	4	Valley to Valley
117	NO	PEAK SIGNALS	AREA	YES	0	valley -to-valley
118	NO	PEAK SIGNALS	AREA	YES	2	valley to valley
119	NO	PEAK SIGNALS	AREA	NO	0	horizontal baseline
120	NO	PEAK SIGNALS	AREA	YES	2	Horizontal baseline
121	NO	PEAK SIGNALS	AREA	YES	0	valley to valley
123	NO	PEAK SIGNALS	AREA	YES	1	valley-to-valley
124	NO	PEAK SIGNALS	HEIGHT	NO	0	xxx
125	NO	PEAK SIGNALS	AREA	NO	0	valley-to-valley
126	NO	PEAK SIGNALS	AREA	YES	2	tangential
127	YES	PEAK SIGNALS	AREA	YES	1	Horizontal baseline
128	NO	PEAK SIGNALS	AREA	xxx	0	valley to valley
129	YES	PEAK SIGNALS	AREA	YES	0	horizontal baseline
130	NO	PEAK SIGNALS	AREA	YES	0	xxx
131	NO	PEAK SIGNALS	AREA	YES	0	xxx
132	NO	Other	AREA	NO	0	horizontal baseline
133	NO	PEAK SIGNALS	AREA	YES	0	tangential
134	NO	PEAK SIGNALS	AREA	YES	4	HORIZONTAL BASELINE
135	NO	PEAK SIGNALS	AREA	YES	0	xxx
136	NO	PEAK SIGNALS	AREA	YES	2	Width, Threshold
138	NO	PEAK SIGNALS	AREA	YES	0	valley-to-valley
139	NO	PEAK SIGNALS	HEIGHT	YES	0	Horizontal baseline

**Table 4f:** Evaluation of the Questionnaire:

Lab ID\ Question	Instructions adequate	Problems with e-Form	Accreditation	Unusual observations
101	YES	YES	NO	At sample D-035
102	YES	NO	YES	
103	YES	NO	YES	
106	YES	NO	YES	
107	YES	NO	YES	
108	YES	NO	YES	
109	YES	NO	YES	
111	YES	NO	YES	
112	YES	NO	YES	
113	YES	NO	NO	
114	YES	YES	YES	-
115	YES	NO	YES	
116	YES	NO	YES	
117	YES	NO	YES	
118	YES	YES	YES	
119	YES	NO	YES	
120	YES	NO	NO	
121	YES	NO	YES	
123	YES	NO	NO	
124	YES	YES	NO	
125	YES	NO	YES	When the standard was analysed as received no DON was detected. After blow-down and reconstitution in mobile phase DON was then detected as expected
126	YES	YES	YES	
127	YES	NO	NO	
128	YES	YES	NO	
129	NO	YES	YES	
130	YES	NO	YES	
131	YES	YES	YES	
132	YES	NO	NO	
133	YES	NO	YES	
134	YES	NO	YES	Blank B-079 and D-104 samples are more similar to maize than to cereal samples
135	YES	NO	NO	
136	YES	NO	YES	
138	YES	NO	YES	
139	YES	NO	YES	

**Table 4g:** Evaluation of the Questionnaire:

Lab ID\ Question	Any other comments?
101	
102	WE USSUALY ANALYSE DON BY ELISA, HPLC METHOD WAS ACCREDITED JUST IN AUGUST THIS YEAR
<b>103 - 111</b>	
112	Why not as XLS or DOC files?
113	The Laboratory is accredited for the analysis of aflatoxins B1,B2,G1,G2 and M1, ochratoxin, nitrates and lead, cadmium and mercury. In above it would be useful if the nature of the matrix of samples was specified.
114	
115	
116	
117	
118	The extraction times were 3 minutes in ultraturrax followed by 60 minutes in orbital shaker
119	
120	
121	
123	The concentration of the sample D-131 was 8,3 ng/g , which is lower than the limit of quantification of the method 20-50 ng/g , depending on the sample type. We only wanted to give all the information we had.
124	
125	
126	
127	We hope to present our method for accreditation at our next Accreditation visit in Spring 2009
128	Methods based on IA-HPLC-UV are not very robust and sensitive. NRL need assistance from CRL for development of an LC-MS/MS-method (maybe in the context of a multiresidue-method together with zearalenone and T-2/HT-2..
129	
130	In Sample D-126 the DON content was < 25 µg/kg. This value could not be entered in the electronic form. The result for the test solution is in ng/ml!! Using the LC-MS/MS method it is very important to have a blank which has the same composition as the samples, because we are dealing with matrix effects. Matrix matched standards were used for quantification. We did see a difference in the three samples, therefore sample D-040 was also analysed by the standard addition. Using this method we found 330.2 µg/kg. Extraction solvent used was MeCN:H2O 84:16 with 1% Formic acid
131	
<b>132 - 139</b>	



**Table 4h:** Evaluation of the Questionnaire:

Lab ID\ Question	Type of analytical problems
<b>101 - 106</b>	
107	LC-MS-MS sensitivity at DON toxin is low.
108	Sample cod D-114 has the same results like sample Blank(B-032); The sample cod 0017 we evaporated to 60 C degrees and reconstitution with the same volume of distillate water;
<b>109 - 123</b>	
124	All samples (see point 7). Additionally, for DON test solution no result could be delivered. Despite several independent attempts, the response for ISTD remained three times lower in test sample than for standards. The ampoule had been opened 6 weeks earlier and the solution stored at -18 degrees meanwhile.
125	
126	Ion suppression in case of D-015 sample. I needed to do a better separation, but the problem was solved.
<b>127 - 139</b>	

**Table 3i:** Evaluation of the Questionnaire:

Lab ID\ Question	Problems with e-Form?
101	It was not possible to put zero after decimal point (it was removed automatically).
<b>102 - 113</b>	
114	It is impossible to fill in results bellow LOQ. Symbols (<) and text are not allowed.
<b>115 - 117</b>	
118	It was not possible to reflect some results as < LOQ because the form only accepts figures (numbers). In question nr 9 has not been possible to reflect the real conditions (see part 23 .- comments))
<b>119 - 123</b>	
124	See above (the result for DON test sample had to marked as 0, although it could not be analysed).
125	
126	The menus did not work
127	
128	impossible to report values as "<x"
129	We use different methods for analysis of DON, but in the form we can choose only one possibility
130	
131	It was not possible to report <RL results for the samples
<b>132 - 139</b>	

**Table 4j:** Evaluation of the Questionnaire:

Lab ID\ Question	Improvmnts?
<b>101 - 127</b>	
128	some contradictions between accompanying letter and reporting sheet
129	To leave more freedom in assessing our recovery
<b>130 - 139</b>	

**Table 4k:** Evaluation of the Questionnaire:

Lab ID\ Question	What is your opinion on the e-Form reporting?
101	Acceptable
102	IT IS GOOD
103	OK but could not submit. Sent as email attachment.
106	Electronic forms are quite good nevertheless, on line links could work better
107	This form is comfortable for use.
108	OK
109	very good
111	ok
112	Somewhat complicated. We have to follow your instruction step by step.
113	It is an easy and quick way to report the results
114	We can imagine that after improvement and changes would be useful for data collection. In such form is confusing , miss-leading and and not friendly.
115	Perfect!
116	It is easy and practical
117	OK
118	OK
119	normal
120	It is useful
121	OK
123	Reporting format was good. But in the instructions it was written "please fill out all fields" and also "The recovery rate will be calculated by us". We left the Recovery field empty.
124	Feasible, although no additional marks to numbers (e.g. text) could be used...
125	easy to use
126	Sometimes it does not work properly
127	Very convenient
128	It's OK!
129	it is imperfect
130	Easy
131	works OK
132	It is good and appropriated
133	OK
134	
135	Excellent
136	It is quite friendly
138	ok
139	Generally OK. Just one small fault on the RESULTS paper: test solution result was supposed to be reported as “ng/ml” – on the form it’s marked as “µg/ml” though (our result is as required in “ng/ml”).

European Commission

**EUR 23787 EN – Joint Research Centre – Institute for Reference Materials and Measurements**

Title: Report on the 2008 Proficiency Test of the Community Reference Laboratory for Mycotoxins, for the Network of National Reference Laboratories, regarding the Determination of Deoxynivalenol in a Cereal Product and a Test Solution

Author(s): J. Stroka, I. Doncheva, A. Breidbach, C. Mischke

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**Abstract**

A proficiency test was conducted by the Community Reference Laboratory for Mycotoxins with 33 European National Reference Laboratories (NRLs) for Mycotoxins and 2 Laboratory from candidate countries, thus a total of 35 participants. Test materials were a deoxynivalenol (DON) solution in acetonitrile and three cereal test materials. Laboratories determined the DON content by either enzyme linked immuno sorbent assay (ELISA), gas chromatography (GC) or reverse-phase high-performance liquid-chromatography (RP-HPLC). One NRL did not report any results.

Applying the Horwitz equation as a basis for the target standard deviation (19% in the case of this proficiency test), 27 out of the remaining 34 laboratories reported values within the z-score limit of 2 after recovery correction of the result for the DON-positive sample. Twenty-five laboratories reported results within a z-score limit of 1. Thus, 79 % of the participating laboratories performed satisfactorily in the proficiency test. No z-scores were calculated for the blank material.



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